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## Evaluation of BiomassBed system in bio-cleaning water contaminated by fungicides applied in vineyard

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BiomassBed is an indirect system designed to collect accidental pesticide spills as well as water remnants of tank sprayers. This system was installed in a vineyard and tested in bio-cleaning water that was contaminated by fungicides in a two-year-field experiment. At the end of each treatment, wastewater of sprayer tanks containing mixtures of cymoxanil, dimethomorph, metalaxyl M, folpet, penconazole, cyprodinil, fludioxonil, azoxystrobin, mandipropamid, pyraclostrobin at different concentrations were discharged into the BiomassBed and repeatedly circulated through an organic biofilter of pruning residues and straw for fungicide adsorption and biodegradation. Water collected was sampled and analysed for fungicide residues, and organic biofilter was analysed to assess fungicide dissipation. Fungicides were removed from water in a range of 92.4–100% of the initial concentration. Metalaxyl M was the least retained and probably desorbed during recirculation of water. However, metalaxyl M residues dissipated in 70 days in collected stagnant water. Abiotic and biotic factors contributing to the disappearance of metalaxyl M in water was hypothesised. Fungicide dissipation in the organic biofilter occurred in a 90-day period with the exception of penconazole that remained at 63.6% and 42.0% in 2008 and 2009, respectively. Owing to the advantageous combination of depuration and bio-degradation processes, fungicides were removed from water in a range of 92.4 to 100%. However more effort is needed to enhance adsorption of mobile fungicides and to achieve a faster degradation of more persistent active ingredients.

**Keywords:** fungicides; organic biofilter; adsorption, dissipation

### 1. Introduction

Pesticide contamination of aquifers is an important unwanted aspect of their use in agriculture. The presence of these active compounds in water bodies may compromise aquatic life and represent a toxicological threat for human beings. The new EC 1107/2009 regulation revises the previous EU 91/414 Directive and introduces the need to adopt a prophylaxis system to prevent the risk of environmental contamination by pesticides used in agriculture with the aim to reach more sustainable agricultural practice. One of the most critical points which may lead to the un-controlled release of pesticides at high concentrations in the environment is attributed to the so-called point source

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contamination represented by accidental pesticide losses during pesticide handling and mixing and by an incorrect discharge of the rinsing water by sprayers.

This type of point contamination can be easily and efficiently prevented by confining this operation to an isolated area that is specially aimed to collect pesticide spills, sprayer tank waste and pesticide residues.

In the last years, the first attempt in this direction was carried out in Sweden [1] by introducing a bio-prophylaxis system called Biobed at farm level that had the aim to receive a small amount of highly concentrated pesticides accidentally lost during the preparation of pesticide mixtures. Biobed is a biological system which firstly adsorbs and thereafter degrades pesticide spills relying on the phenol-oxidase activity of white-rot fungi grown on a suitable mixture of soil-peat-straw [2]. On the basis of Biobed principles, different bio-prophylaxis systems have been designed and studied in various laboratories and farms in several other EU countries [3,4]. Many of these systems have been designed to treat both accidental pesticide spills as well as different volumes of pesticide-contaminated water deriving from cleaning of tanks at the end of field treatments. In a recent workshop, the 3rd European Biobed Workshop [5], many of the results obtained regarding the performance of these systems in both EU and non-EU Countries, were presented. Till date, Sweden is the leading country in Northern Europe with about 1500 Biobeds installed at farm level.

The need to introduce such systems in Mediterranean countries, where the length of crop growing season and the variety of crop yields lead to the use of a high amount of several pesticides, is strongly recommended. A specific case is represented by viticulture which is an important and very widespread agricultural activity in Southern Europe and is based on an intensive use of pesticides, especially fungicides. Depending on their chemical classes, fungicides may represent active substances persistent in soil [6] and being often detected in stream water near vineyards [7,8].

In Italy, at present, six experimental bio-purification systems at farm level are under study. One of them is called BiomassBed which is installed at the experimental farm of Università Politecnica delle Marche as part of the ARTWET project [5]. BiomassBed was designed to collect and to biologically treat both concentrated and diluted pesticide residues. It is an indirect system where all pesticide spills or water remnants of sprayers are collected in a buffer tank and allowed to re-circulate through an organic biofilter for pesticide adsorption and biodegradation. The final destination of the exhausted biofilter is still a controversial point since it is considered a special waste because of the presence of harmful substances. Indeed, one of the most important goals is to obtain a complete dissipation of fungicides within the biomixture to allow the safe disposal of the exhausted biofilter. In order to allow maximum pesticides biodegradation and the presence of a final amount of pesticide residues compatible with an acceptable environmental risk, the biomixture should favour the establishment of an active microbial community that is able to degrade pesticides at high concentration [9]. The Swedish Biobed mainly relies on the fungal community, especially lignin degrading fungi and their phenoloxidase activity, for the cometabolic degradation of pesticides [2,10–13], while the French Phytobac relies on the bacterial community to pesticides degradation with the aim to obtain complete mineralization of the molecules [14]. Overall, several different biomixtures were studied to assess their potential in the sorption and biodegradation of pesticides [9,15–23]. Depending on the materials that were locally available, the biomixtures had different compositions, presumably characterized by different consortia of colonizing microorganisms and received different pesticide mixtures which in turn diversely affect the microbial metabolic activity.

This is the reason why the main chemical and biochemical processes that regulate sorption and degradation of pesticides loaded in the biomixtures and the interactions with the native microbial community are still not sufficiently clear.

Under Southern Europe climatic conditions, biomixtures constituted of green composted material and added with fresh ligninocellulosic material from local agricultural activity were recognized to perform well in pesticide sorption and biodegradation [9,15,18–20,23].

A field experiment was carried out with the aim to test the bio-cleaning efficiency of BiomassBed against fungicides applied in vineyards for two years of field treatments. A biomixture of pruning residues and straw was considered as biofilter for the adsorption and bio-degradation of cymoxanil, dimethomorph, metalaxyl M, folpet, penconazole, cyprodinil, fludioxonil, azoxystrobin, mandipropamid and pyraclostrobin. Biodepuration efficiency was expressed as percentage of pesticides retained against the total amount of pesticides discharged into BiomassBed during the spraying seasons. Accumulation and dissipation of each active ingredient was also followed in the biofilter for both first and second year of experimentation, in order to assess the time required for pesticides dissipation and provide a safe disposal of the used biofilter.

## 2. Experimental

### 2.1 Site of experimentation

A BiomassBed was installed at the experimental “P. Rosati” farm of Università Politecnica delle Marche, Agugliano (AN), Italy, 43°32'48"N, 16°22'00" at about 80 m above sea level. The bio-purification system was installed in March 2008, and it was tested in bio-cleaning water contaminated by fungicides applied in a 2.2 ha vineyard during the grapevine season of 2008 and 2009. The experimental area (Figure 1) was equipped with a concrete area (6 m × 3 m) where all the operations of filling and washing of the tanks used for the field treatments, were performed. A drain was used to transport all the pesticide remnants to a buffer tank. Fungicide residues, derived mainly from the volume of the residual formulates, varying from 6 to 15 L were directly discharged into the drain. Rinsing water (about 100 L), sprayed to clean up the internal and the external parts of the atomizer, was collected by applying fenders upon the nozzles and then conveyed to the drain (Figure 1a).

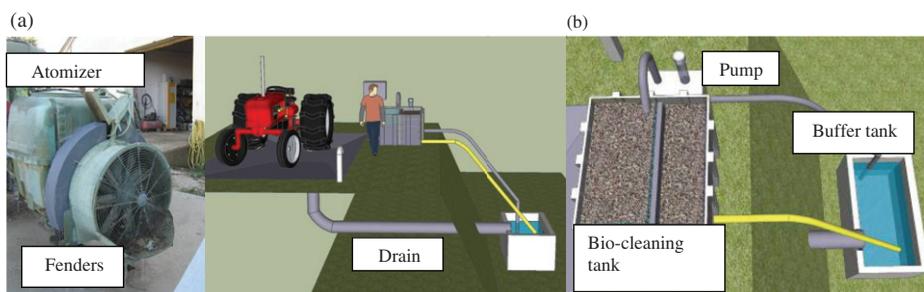


Figure 1. Description of the experimental site: (a) atomizer with fenders (b) BiomassBed system as outline and top view.

Table 1. Main chemical properties of the organic substrates and bio-filter.

Organic substrates	pH (H <sub>2</sub> O)	C <sub>org</sub> (%)	C <sub>tot</sub> (%)	N <sub>tot</sub> (%)	C <sub>tot</sub> /N <sub>tot</sub>
PR2	7.21	40.7	49.3	1.55	31.6
PR5	7.05	21.8	31.1	2.17	14.3
WST	7.14	32.9	42.4	2.07	20.5
Bio-filter	7.13	28.8	37.0	2.06	18.0

PR2 = pruning residues after two years of degradation.

PR5 = pruning residues after five years of degradation.

WST = wheat straw.

## 2.2 Organic biofilter

The biofilter consisted of a biomixture of pruning residues from an orchard of apples, peaches, apricots, cherries, olives and grapevine left to degrade naturally in an open field for 2 (PR2) and 5 (PR5) years, respectively, and fresh wheat straw (WST) (Table 1). PR2 and WST were cut at 3–6 cm to enhance their density and adsorption capacities. The final composition of the biomixture was 40% (v/v) of PR2, 40% (v/v) of PR5 and 20% (v/v) of WST. Each organic material was measured volumetrically, manually mixed into a plastic bag to obtain a homogeneous biomixture with a density of 0.431 g cm<sup>-3</sup> and a water holding capacity of 760 mL kg<sup>-1</sup>.

## 2.3 Fungicides tested

Table 2 shows the active ingredients applied in the vineyard during 2008 and 2009, the residual amount of each fungicide discharged into the BiomassBed system at each treatment, and days between each treatment. To avoid accumulation of copper, which could lead to an inhibition of the metabolic activity of the microbial community of the biofilter, the inorganic fungicides applied in the vineyard during both years of treatments were not disposed into BiomassBed. The main chemical and environmental fate properties of the active ingredients tested and analysed in water and in the organic-biofilter are reported in Table 3 [24].

## 2.4 BiomassBed system

The bio-purification system is illustrated in Figure 1b. The BiomassBed system is equipped with a buffer tank (1000 L capacity) that collects all the residuals of concentrated as well as diluted pesticides. The buffer tank is connected by a pipe to a bio-cleaning tank (600 L capacity) in which pesticides are retained and biodegraded. The presence of a pump allows circulation of contaminated water from the buffer tank to the bio-cleaning tank and thus throughout the biomixture for pesticide sorption. The bio-cleaning tank is divided into two sections. The first section contains inert material such as coral pebbles, which pre-purifies the water and the second section contains eight plastic boxes (two piles of four boxes), each filled with 7.5 kg of the organic substrate chosen to act as biofilter. In total 60 kg of organic substrate were placed in BiomassBed.

At the end of each field treatment in the vineyard, residues of fungicides were washed out from the internal and external parts of the atomizer (as reported above) and collected

Table 2. Field treatments of 2008 and 2009: organic fungicides applied, date of application, residual amount of active ingredients and days between treatments.

Treatments	Organic fungicides mixtures	Residues in washing water (mg)	Time between treatments (d)	
2008				
1°	30 April	CYM	100	0
2°	15 May	MX+PC	740+350	15
3°	27 May	MX+PC	730+420	10
4°	6 June	MX+FP+PC	1100+3500+410	9
5°	9 June	MX+FP+PC	70+540+23	3
6°	20 June	AZX+FP	2000+1100	10
7°	16 July	DM	2200	24
8°	25 July	CYP+FLU	3200+2100	8
2009				
1°	28 April	CYM	1900	0
2°	6 May	DM	2200	8
3°	14 May	MN+PC	680+240	7
4°	26 May	MN+PC	1300+440	12
5°	5 June	MX+PC	1000+500	9
6°	16 June	MN+MX	670+520	10
7°	30 June	PYR	1100	13
8°	22 July	CYM	1300	21
9°	28 July	MN	1600	6

CYM = cymoxanil; PC = penconazole; MX = metalaxyl M; AZX = azoxystrobin; FP = folpet; DM = dimetomorph; CYP = cyprodinil; FLU = fludioxonil; MN = mandipropamid; PYR = pyraclostrobin.

Table 3. Some chemical and environmental properties of fungicides tested [24].

Pesticide	Solubility (H <sub>2</sub> O) (mg L <sup>-1</sup> )	Koc (mL g <sup>-1</sup> )	DT50 (days)
CYM	780	46	0.7
PC	73	2205	197
MX	26,000	660	39
AZX	6.7	423	70
FP	0.8	304	4.7
DM	29	348	57
CYP	13	1706	45
FLU	1.8	75,000	125
MN	4.2	847	17
PYR	3.1	898	20

CYM = cymoxanil; PC = penconazole; MX = metalaxyl M; AZX = azoxystrobin; FP = folpet; DM = dimetomorph; CYP = cyprodinil; FLU = fludioxonil; MN = mandipropamid; PYR = pyraclostrobin.

in the buffer tank through the drain. From the buffer tank, contaminated water was pumped into the bio-cleaning tank several times, allowing leaching through the biofilter and then collected again in the buffer tank.

The collected water remained in the buffer tank during the entire period of the spraying season. Both bio-cleaning and buffer tanks were covered during the period between each treatment which are reported in Table 2.

The retention capacity of the biofilter against each active ingredient was evaluated by sampling the water in the buffer tank before and after each depuration cycle. In the case of repeated application (i.e. penconazole, metalaxyl, mandipropamid, etc.), the initial amount of the active ingredient in the buffer tank at each depuration cycle has to be considered as the sum of the amount derived from the previous cycle and the amount loaded in the current cycle.

After the depuration cycles related to field treatments, two further depuration cycles were performed for both years of experimentation without any addition of fungicides in order to enable complete water cleaning.

In 2008, since after these two depuration cycles metalaxyl M was not completely removed, water in the buffer tank was left to stand for another 70 days and then sampled again to measure metalaxyl M residues.

### **2.5 Fungicide dissipation study**

At the end of each year of various treatments, the boxes containing the organic biofilter were removed from BiomassBed and placed in an open surface under environmental temperature conditions. The moisture level of the biomixture was maintained by regularly adding water at about 60% of its water holding capacity. During a 90-day period, the biomixture was sampled and analysed for fungicide residues.

The initial concentration of each fungicide on the biofilter was that measured on the biofilter on 25 July (2008) and 28 July (2009).

### **2.6 Analysis of fungicide residues in water and in the organic biofilter**

Aliquots of 50 mL of water sampled were treated with 50 mL of chloroform which was left to evaporate in a rotary vacuum evaporator before re-dissolving fungicide residues in 1 mL of methanol and to analytically identifying and quantifying them by high performance liquid chromatography (HPLC). Analyses of residues in water were performed in duplicate.

An amount of organic biomixture corresponding to 50 g of dried matter was extracted with 50 mL of a solution of methanol and water (80:20 v/v). They were shaken for 1 hour on an orbital shaker, centrifuged and filtered. An aliquot of 25 mL was then partitioned three times in 50 mL of chloroform, left to dry in a rotary vacuum evaporator. Fungicide residues were re-dissolved in 1 mL of methanol and analysed in HPLC. The analysis of residues in the biomixture was performed in triplicate.

Fungicide residues were identified and quantified by applying a multiresidue method using HPLC consisting in a mobile phase of methanol and pure water (60:40, v/v), a flow rate of 1 ml min<sup>-1</sup> and detection wave length of 207 nm.

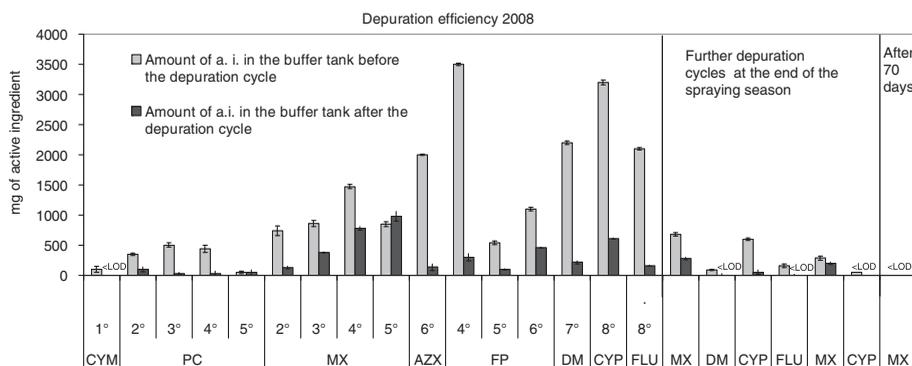
The HPLC system was a Spectra SYSTEM P 4000, equipped with a variable wavelength UV detector and a Supelcosil C18 column 5 µm × 4.6 mm i.d. × 230 mm

in length. Under these conditions, the linearity of detector response was determined on a set of eight standard concentrations ranging from 5 to 100  $\mu\text{g L}^{-1}$  of methanol. Pesticide recoveries had an average of 70% (for the biofilter) and 95% (for water). Retention times were: cimoxanil, 5.3 min L, metalaxyl M 6.3 min L, azoxystrobin 9.1 min L, pyraclostrobin, 9.6 min L, mandipropamid 10.8 min L, dimetomorph 12.4 min L, fludioxonil 20.3 min L, cyprodinil, 27.8 min L, penconazole 28.9 min L, folpet 33.6 min L. Depending on the active ingredient tested, LOD (limit of detection) ranged between 0.25 to 0.75  $\text{ng } \mu\text{L}^{-1}$ . LOQ (limit of quantification) ranged between 5 and 10  $\mu\text{g L}^{-1}$  in water and from 15 to 25  $\mu\text{g Kg}^{-1}$  in the biomixture.

### 3. Results and discussion

Depuration efficiency of organic biofilter versus fungicides loaded into BiomassBed during different treatments in 2008, for each depuration cycle (1°–8°), is reported in Figure 2. Cymoxanil, azoxystrobin, dimetomorph, cyprodinil and fludioxonil were loaded once at the amounts of 100, 2000, 2200, 3200 and 2100 mg, respectively. After the contaminated water through the organic biofilter had circulated, cymoxanil amount was below the limit of detection while final amounts of 180, 220, 610 and 160 mg of azoxystrobin, dimetomorph, cyprodinil and fludioxonil, were found, respectively. The depuration efficiency estimated for these fungicides varied from 81% for cyprodinil to 100% for cymoxanil.

Folpet was loaded three times into BiomassBed for a total amount of 5140 mg, and 463 mg were found in the buffer tank after the circulation of water, which means that the depuration efficiency of the biofilter against this active ingredient was 91%. Penconazole was loaded four times into the BiomassBed. The total amount was 1203 mg, and at the end of the applications, 50 mg were detected in the buffer tank showing a depuration efficiency of 96%. A different behaviour was observed for metalaxyl M. A total amount of 2640 mg of metalaxyl M was loaded into the BiomassBed in four applications, and a final amount of 980 mg was still detected in the buffer tank after water circulation. The biofilter



CYM=cymoxanil; PC=penconazole; MX=metalaxyl M; AZX=azoxystrobin; FP=folpet; DM=dimetomorph; CYP=cyprodinil; FLU=fludioxonil; 1°–8° depuration cycles; LOD = limit of detection

Figure 2. Depuration efficiency of organic bio-filter versus fungicides loaded into the BiomassBed during treatments of 2008, for each depuration cycle (1°–8°).

showed the lowest depuration efficiency against this active ingredient since only 63% of the total metalaxyl M loaded was retained.

During the spraying season 2008, results of water bio-purification showed that sorption efficiency of the bio-mixture varied with fungicides discharged. Among the more mobile fungicides, cymoxanil represented an exception being adsorbed more than the well-retained fungicides such as fludioxonil (Table 4). In fact, despite the lowest Koc (Table 3) value and the scarce tendency to be adsorbed on organic matter, biomixture showed 100% of bio-depuration efficiency against cymoxanil. In accordance with Fait *et al.* [25] this behaviour was attributed to the low amount of cymoxanil discharged into the biomixture more than to the mobility properties of the molecule. Indeed, during the first year of treatments, cymoxanil was discharged at a very low amount if compared to both mobile and less mobile fungicides (Table 2). As such, it is reasonable to hypothesize that the biomixture was able completely to retain cymoxanil by lowering its concentration in water under the limit of detection.

Another remarkable behaviour was observed for metalaxyl M. With its last application, a very low amount (70 mg) of metalaxyl M was discharged into BiomassBed. Despite this fact, the concentration of the fungicide in the water of the buffer tank tended to rise. This tendency was attributed to mobility and, especially to the high solubility of this active ingredient itself. It is possible that the combination of these two properties caused weak adsorption of the molecule on the organic biofilter. In previous studies, Vischetti *et al.* [15] and Karanasios *et al.* [9,23] showed that metalaxyl is one of the less retained pesticide when leached throughout organic biomixtures for bio-depuration systems. Adsorption-desorption studies under batch-equilibrium conditions have showed that an average value of 30% of metalaxyl already sorbed on the organic biomixture can be easily desorbed [20]. Furthermore, the level of the hydraulic flow of contaminated water may have significantly affected the sorption of pesticides characterised by intermediate mobility. Indeed, among other pesticides, De Wilde *et al.* [26] showed that metalaxyl was retained less by the organic biomixture when the contaminated water was leached at the highest flux.

Table 4. Biodepuration efficiency of the BiomassBed system during the spraying season 2008 and 2009.

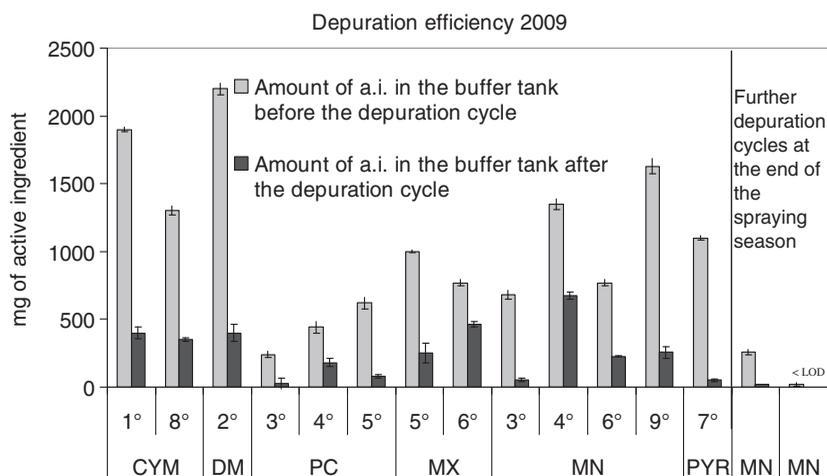
	2008 % of active ingredient retained	2009 % of active ingredient retained
More mobile fungicides		
CYM	100	89
AZX	91	–
DM	90	82
FP	91	–
MX	63	69.6
Less mobile fungicides		
CYP	81	–
FLU	92.4	–
PC	96	93.2
MN	–	94
PYR	–	95.5

Thus, both the chemical properties of metalaxyl M as well as the settled pressure of the BiomassBed pump which regulates the flow rate of contaminated water, could have contributed to a lower retention efficiency of the biomixture against metalaxyl M. Moreover, the continuous circulation of water through the biofilter may have caused the de-sorption of a part of the amount of the active ingredient already sorbed [20] bringing it back into the water of the buffer tank and increasing in concentration.

The depuration efficiency of organic biofilter versus fungicides loaded into BiomassBed during the treatments in 2009 for each depuration cycle (1°–9°), is reported in Figure 3. Dimetomorph and pyraclostrobin were applied only once and a total amount of 2200 and 1100 mg respectively was loaded into Biomassbed. The circulation of contaminated water lowered the presence of these fungicides to a final amount of 400 mg (dimetomorph) and 50 mg (pyraclostrobin) leading to an estimation of a total depuration efficiency of 82.0 and 95.5%, respectively. Metalaxyl M and cymoxanil were applied twice at 1520 mg and 3200 mg, respectively. Metalaxyl M remained at the amount of 462 mg in water with a depuration efficiency of 69.6% showing the same behaviour as in the previous year, confirming the hypothesis that circulation of water does not allow the highest adsorption of this molecule. On the contrary, it leads to de-sorption of a part of the active ingredient retained as consequent mobility and high solubility properties of this molecule.

In contrast with results obtained during the first year of experimentation, cymoxanil remained at 350 mg in water with a depuration efficiency of 89% confirming that the highest sorption efficiency observed during the first year of experimentation was more due to the low amount of the active ingredient discharged than to the mobility properties of the molecule.

Penconazole was applied three times for a total amount of 1180 mg loaded into the BiomassBed, and at the end of all penconazole applications, a final amount of 80 mg was detected in the water of the buffer tank leading to a final depuration efficiency of 93.2%.



CYM=cymoxanil; DM=dimetomorph; PC=penconazole; MX=metalaxyl M; MN=mandipropamid; PYR=pyraclostrobin; 1°–9° depuration cycles; LOD = limit of detection

Figure 3. Depuration efficiency of organic bio-filter versus fungicides loaded into the BiomassBed during treatments of 2009, for each depuration cycle (1°–9°).

The last four field treatments involved mandipropamid. On the whole, 4250 mg of this fungicide were loaded into the BiomassBed which lowered the presence of mandipropamid to 255 mg with a depuration efficiency of 94%.

The sorption efficiency of the biomixture against fungicides loaded during 2009 was in the same range as that observed during 2008. Overall, an average of 80.2% of the more mobile fungicides against an average of 94.2% of the less mobile molecules was retained by the biomixture (Table 4).

During both years of experimentation, peaks in concentration of most of the pesticides loaded into BiomassBed were found in the water of the buffer tank immediately after the depuration cycle that followed the discharge of residual fungicide mixture used in the field treatment. In fact, after eight depuration cycles in 2008 and nine depuration cycles in 2009, the only molecules detected in the water of the buffer tank were those that were discharged at the last. These results clearly indicate that all the active ingredients loaded previously were reasonably sorbed and/or dissipated by the biomixture during the period of treatments which lasted 86 and 90 days in 2008 and 2009, respectively. To complete the bio-cleaning of the water from the fungicides that were applied last, two further depuration cycles were performed during the two years of experimentation.

In 2008, the bio-purification of water was not had completely successful as regards metalaxyl M as 200 mg (Figure 2) of it had still remained in water. However, 70 days after the last depuration cycle, it was not possible to detect any residue of metalaxyl M in the stagnant water of the buffer tank. Since metalaxyl M is quite a persistent fungicide in water [24], it was hypothesized that a combination of abiotic factors, such as temperature and light, as well as biotic factors such as presence of dissolved organic matter and microflora of the organic biofilter that ended in the water of the buffer tank, may have helped in the dissipation of metalaxyl M. Microbial degradation in the aquatic system carried out by both bacteria (*Pseudomonas* sp) and fungi (*Aspergillus niger*, *Cladosporium herbarum* and *Penicillium* sp) has been proved to occur in four weeks of experimentation [27]. Further, a fast degradation trend of metalaxyl in water was reported by Yao *et al.* [28] who found that in special conditions such as those under UV irradiation and in the presence of some photodecomposition activators, more than 70% of metalaxyl in water degraded in 5 days.

With the exception of metalaxyl M, BiomassBed system showed good performance in bio-cleaning the water that was contaminated by fungicides applied in the vineyard. The results obtained after two years of experimentation under real field operative conditions are in accordance with those reported in literature for similar bio-prophylaxis systems preventing water body contamination by pesticides used in agriculture [3].

In order to assess the time required to dissipate sorbed fungicides, the biomixture was analysed for fungicide residues immediately after the last depuration cycle of water and monitored for fungicide residues for 90 days. The initial amounts of fungicides detected in the biomixture are the results of the accumulation and dissipation phenomena that occurred during the spraying season of 2008 and 2009.

Figures 4 and 5 report the results of fungicide dissipation for 2008 and 2009 monitored for 90 days. In 2008, six of the eight fungicides loaded were detected in the organic biofilter, which were metalaxyl M, penconazole, azoxystrobin, dimetomorph, cyprodinil and fludioxonil, highlighting that cymoxanil and folpet dissipated in the biomixture during the spraying season lasted for almost three months. In 2009, four out of six fungicides were detected in the organic biofilter, which were dimetomorph, mandipropamid, penconazole and pyraclostrobin, while cymoxanil and metalaxyl M dissipated within the period of treatments.

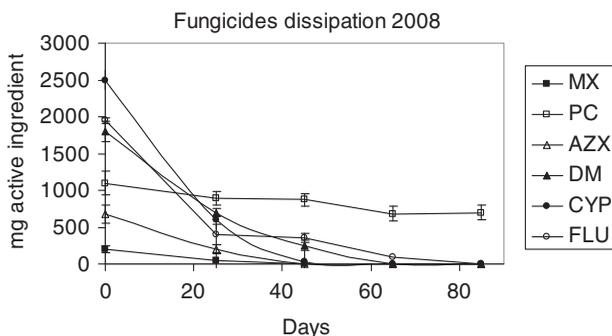


Figure 4. Dissipation of fungicides in the organic bio-filter during 2008 in a 90-day experiment.

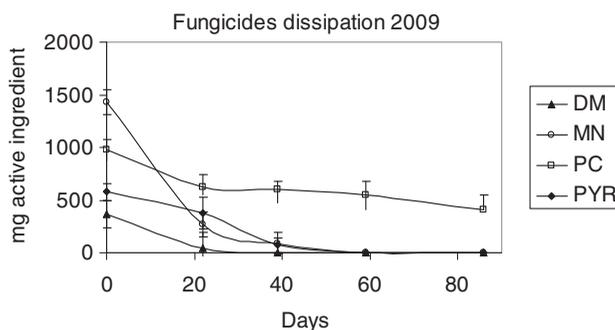


Figure 5. Dissipation of fungicides in the organic bio-filter during 2008 in a 90-day experiment.

In accordance with the results found by Fait *et al.* [25] a complete dissipation of cymoxanil was achieved since this molecule was detected neither in water nor in the biomixture at the end of both years of experimentation. The fast dissipation of cymoxanil was somehow expected and it can be easily explained by considering the short half-life of the molecule itself which, in soil, is of 0.7 days (Table 3). The same conclusions can be made for folpet ( $DT_{50} = 4.7$ ), that despite being loaded at the highest total amount of 5140 mg (Table 2) owing to a very short half life, was not detected in the biomixture.

At the end of the spraying season in 2008, metalaxyl M was detected at a very low amount and no residues were detected in the biomixture at the end of the spraying season in 2009. Metalaxyl is considered as moderately persistent fungicide (Table 3). It has been proved that repeated application [19] of the biomixture as well as the use of metalaxyl-primed material [26] as constituent of the biofilter can induce an enhanced degradation of metalaxyl by reducing the half-life period of the fungicide. The different mixtures and amount of fungicides discharged could have differently influenced the metabolic activity of the microbial community leading to a slightly slower dissipation of metalaxyl M during 2008 in comparison to that of the 2009 spraying season. However, dissipation of fungicides detected in the biomixture mostly occurred within 40–80 days, in 2008 (Figure 4) and within 20–60 days in 2009 (Figure 5), with the exception of penconazole where 63.6% and 42.0% of the initial amount was still present in 2008 and 2009, respectively.

Triazoles are considered to be persistent molecules as far as their degradation in soil [24] is concerned, with half-life values of 197 days at 20°C in laboratory. In accordance with the results obtained by Fogg *et al.* [29] who found a low rate of degradation of epoxiconazole, penconazole turned out to be more persistent in the biomixture in comparison to the other fungicides that were applied. On the other hand, fludioxonil is also a recalcitrant molecule with a very high affinity for the organic matter ( $K_{oc} = 75000 \text{ mL g}^{-1}$ ). In this specific case, it was not possible to determine extractable residues only after about 60 days, which suggests that this fungicide may have undergone to both microbial degradation as well as an “aging” sorption process, as it is a molecule that is irreversibly bound to the organic biomixture.

With the exception of penconazole, the results of fungicides dissipation in the tested biomixture were in the same range as those observed by Karanasios *et al.* [9] in similar biomixtures constituted by green composted and fresh lignocellulosic material; the authors affirm that in all the biomixtures tested it was mostly difficult to correlate pesticides degradation with general biochemical parameters, such as FDA hydrolytic activity and/or basal respiration as well as specific enzymatic activities, such as MnP and Lac phenoloxidase. For this reason, in the present study, fungicides dissipation that occurred in the biomixture was considered as the result of the activity of a consortium of both fungi and bacteria.

Fast dissipation of active ingredients is needed to safely dispose of the organic biofilter in the environment for example as an organic amendment. The environmental risk represented by the exhausted biofilter is still a controversial point. Even if dissipation of pesticides may occur more or less rapidly in the biofilter, to date it is not sufficiently clear which micro-organisms are mainly involved, what dissipation pathways are there, what metabolites are formed and what is their toxicity. As such, it is still difficult to ensure the chemical safety of the exhausted organic biofilter in the environment. On the other hand, bio-prophylaxis systems based on the use of organic biomixture are important because of the microbial activity of such substrates which may lead to the bio-remediation of toxic compounds.

#### 4. Conclusions

A BiomassBed system working with a biofilter consisting of a biomixture of pruning residues and straw performed very well in terms of biocleaning water containing fungicides applied in vineyards.

Circulation of contaminated water through an organic biofilter during a spraying period allowed to obtain an almost complete depuration from all the fungicides tested. Owing to its high mobility and solubility, metalaxyl M was the least retained fungicide, and it has been shown to be desorbed during water re-circulation.

Dissipation of fungicides in the organic biofilter occurred rapidly with the exception of penconazole that degraded relatively slowly.

Due to the advantageous combination of depuration and bio-degradation processes, fungicides were removed from water in a range of 92.4–100%. However, more effort is needed to enhance adsorption of mobile fungicides by enhancing the contact time period between mobile fungicides and biomixture and by achieving a faster degradation of more persistent active ingredients.

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